THE MOLTING CYCLE OF THE SPINY LOBSTER, PANULIRUS ARGUS LATREILLE. II. PRE-ECDYSIAL HISTOLOGICAL AND HISTOCHEMICAL CHANGES IN THE HEPATO-PANCREAS AND INTEGUMENTAL TISSUES[1]

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The external manifestations of an approaching molt and of molting itself are the result of more basic physiological phenomena related to growth. Growth in the arthropods is markedly conditioned by the properties of the exoskeleton, which are in turn conditioned by the epidermis and other tissues. In the Crustacea, as in other arthropods, growth is cyclical, periods of comparative rest alternating with periods of activity. These cyclic periods of growth are accompanied by cyclical changes in the epidermis, sub-epidermal tissues and the hepatopancreas. The present paper is a study of the pre-ecdysial histological and histochemical changes in the integumental tissues and the hepatopancreas of *Panulirus argus* Latreille.

Materials and Methods

Animals

Male and female spiny lobsters ranging in carapace length from 80–89 mm. were obtained and handled as previously described (Travis, 1954).

Designation of stages in the molting cycle

In a study of the molting cycle, some method of designating the essential stages is necessary. Two main methods have been resorted to, namely: 1) indication of actual time periods, and 2) indication of morphological characters of skeleton and tissues. The first of these methods offers certain advantages. By this method, it is possible to determine the length of the intermolt periods in each size group and to determine the duration of existing morphological characters of the skeleton at each season of the year. In this manner any animal in the laboratory can, from its previous laboratory record, be placed in the proper stage of the molting cycle either by days following molt or by the morphological characters of the skeleton. The second method consists of breaking down the molting cycle into four major stages, A, B, C, D, and subdividing each of these stages, according to Drach (1939).

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Its major advantage is that it provides a means of grouping animals into the major stages of the molting cycle without reference to the interval of time over which such stages extend. Another advantage of Drach's method is that, regardless of age or size animals of corresponding stages can be compared even though the duration of these stages varies with size and season.

Both methods should be used since they are supplementary and necessary for interspecific comparison. The following time intervals for the molting cycle of *Panulirus* refer to animals of 80–89 mm. carapace length. The length of the entire intermolt period in the summer months is usually 65–70 days (Travis, 1954).

Stage A—Stage immediately following molt. The exoskeleton is of the consistency of a soft membrane. The animals do not feed. The duration is about 24 hours.

Stage B—Preliminary hardening of the skeleton occurs, which attains the consistency of parchment. The carapace is rigid in certain regions while the branchiostegites remain soft. No feeding occurs. The duration is from one day postmolt through the fifth or sixth day.

Stage C—The skeleton is entirely hardened but continues to thicken during a good portion of the period. This is a period of active feeding and is the longest of

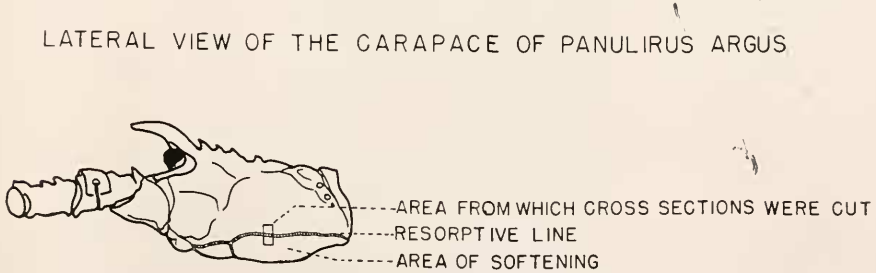


FIGURE 1. Lateral view of the carapace of *Panulirus* to show the area from which sections of the integument were cut.

the cycle, lasting from approximately the seventh to the fifty-first day following molting, or a total of about forty-four days. During late Stage C, the membranous layer and the principal layer are completed. The term "intermolt animal" will be applied to animals with a fully developed skeleton, a condition which falls approximately midway in days between two ecdyses, *i.e.*, 28–35 days following one molt and preceding the next.

Stage D—The new future skeleton is progressively constructed under the old, while the old is gradually broken down by resorption of both mineral and organic constituents. The period lasts ten to fourteen days during which the animals do not feed.

Histological and histochemical methods

For the histological and histochemical studies pieces of skeleton were removed from the carapace of *Panulirus* (Fig. 1) approximately five, three, and one days before a molt. Also, pieces of integument were removed on each of eight consecutive days following molt (a study of which will be reported in a subsequent paper) and from intermolt animals (late Stage C). By removal of pieces of skele-

ton during the pre-ecdysial period (late Stage D) the extent of the resorption in the endocuticle could be detected.

The right posterior lobe of the hepatopancreas was removed at approximately three days and one day before molt, on each of seven consecutive days following molt, and from animals in late Stage C.

All integumental tissues were embedded in celloidin and cut at 10 μ. The posterior lobe of the hepatopancreas was embedded in paraffin and cut at 8 μ. One to three animals were used to represent each of the days mentioned above. Portions

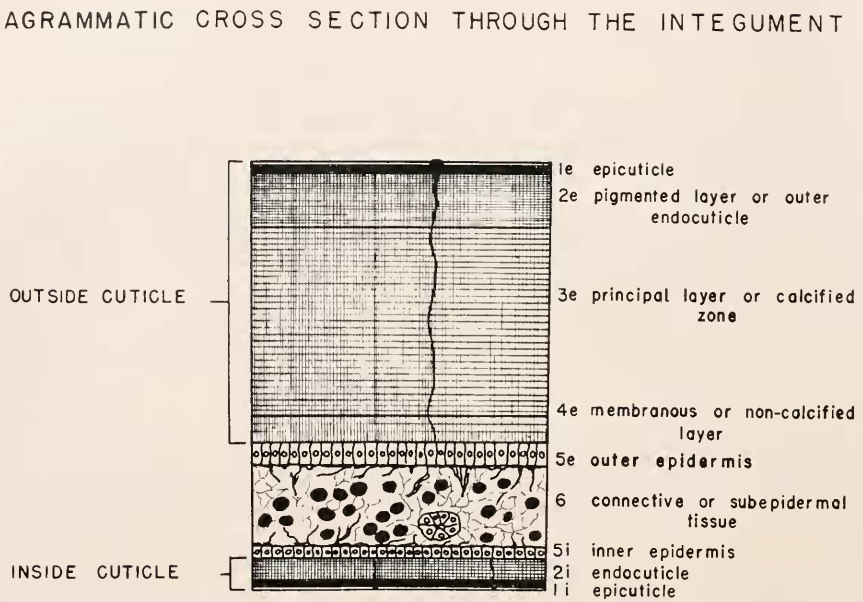


FIGURE 2. Diagram of a section through the integument of the branchial region (late Stage C). Note outside cuticle consisting of four distinct layers with its epidermis and inside cuticle consisting of two distinct layers with its epidermis. The inside cuticle borders the outer periphery of the gill chamber. The spongy sub-epidermal connective tissue is sandwiched between the outer and inner epidermis. The large black oval cells represent reserve cells.

of skeleton and hepatopancreas fixed in Helly's and alcoholic Bouin's fluid were stained by the following methods:

1. Mallory's triple stain.
2. Periodic acid-Schiff (PAS) of McManus, as described by Lillie (1948).
3. Bensley and Bensley's method (1938), demonstrating mucins by means of toluidine blue. The thiazine dyes have three absorption bands: alpha, beta, and gamma. The alpha form is orthochromatic (blue), whereas beta metachromasia (violet) can be caused by highly polymerized carbohydrates or by phosphate-containing compounds. Substances which give gamma metachromasia (red or pink) are usually acid mucopolysaccharides (Pearse, 1953). Since chitin is considered to be a neutral mucopolysaccharide (Meyer, 1938) and is closely associated with protein in the arthropod cuticle, since there is no real histochemical distinction

possible between neutral mucopolysaccharides and mucoprotein (Pearse, 1953), and since in early stages of skeletal deposition gamma metachromasia is exhibited, the pink color produced by the dye probably indicates the presence of muco- or glycoproteins.

For the detection of calcium deposits, portions of the skeleton were fixed in nine parts of 95% alcohol and one part of 40% formaldehyde and were stained with the following:

1. Mallory's triple stain.
2. Schmorl's purpurin (Lillie, 1948).
3. Alizarin red S (Manigault, 1939).
4. Von Kossa's method (Lillie, 1948). Before following this procedure, tissues were washed in 5% aqueous KNO_3 for five minutes or more to remove some of the chloride present.
5. Microincineration (Scott, 1933) was used to confirm the presence of calcium deposits detected by the stain mentioned above.

The hepatopancreas was also fixed in formol-alcohol and was stained with Mallory's triple and Alizarin red S, the latter stain being used to detect calcium.

Portions of the skeleton and hepatopancreas were fixed in cold 80% alcohol, and alkaline phosphatase was determined by the method of Gomori (1941). Control sections (Fig. 32) were made, using distilled water without added substrate during incubation.

Portions of the hepatopancreas which were fixed in 10% neutral formalin were embedded in carbowax (method of Blank and McCarthy, 1950), cut at 10 and 15 μ and stained for lipids with Sudan black B.

In sections of exoskeleton from premolt animals where the resorptive line becomes apparent, the folding, and in some cases the loss of the old cuticle made detection of this line impossible even in celloidin sections. Therefore, sections made by hand or whole mounts were used to study resorption in this area.

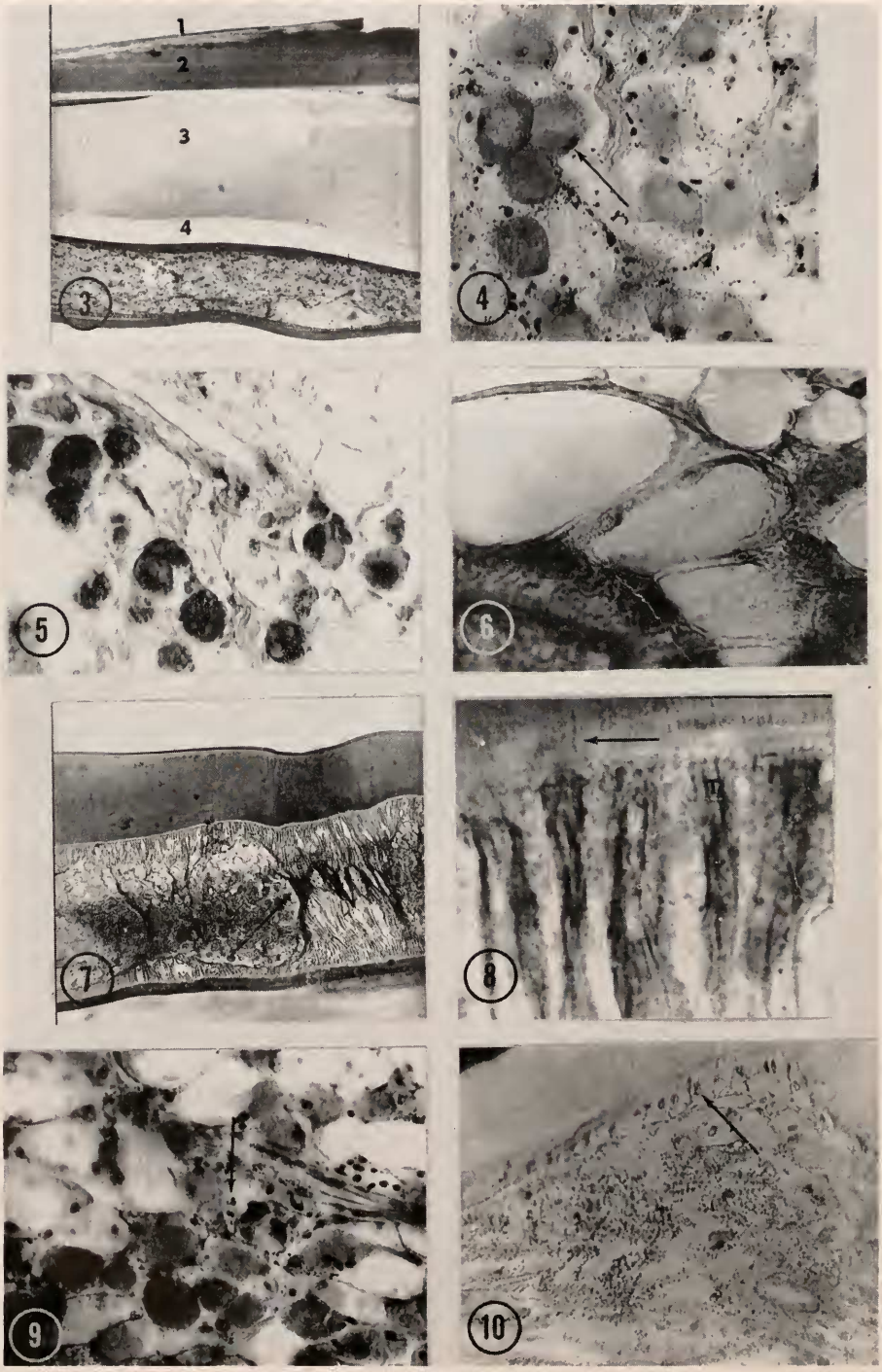
Observations

A. The intermolt animal (late stage C)

1. *The integument and integumental tissues*

a. *Tissues*

In pieces of exoskeleton from the lateral portion of the carapace the attached tissues are bordered by two integuments or cuticles as indicated in Figures 2 and 3. The inner integument, which forms a boundary for the gill chamber, is approximately 1/20 the thickness of the outer one (Fig. 3). Underlying each of these integuments is a layer of columnar epidermal cells with centrally located nuclei, and between these two layers is sub-epidermal connective tissue of a loose spongy type (Figs. 2, 7). Large oval reserve cells (Figs. 4, 5, 9), which Cuénot (1893) called "protein reserve cells" in *Astacus fluviatilis* and which resemble Type 1 Leydig cells (Kükenthal, 1926–1927) and Langer's vesicles of Mollusca (Bronn, 1896–1897), constitute by far the most predominant type of cells in this tissue. They appear as vesicular cells with a capsule-like envelope of cytoplasm containing a peripheral nucleus. These reserve cells vary in appearance during the molting



FIGURES 3–10.

cycle, binding or storing large amounts of polysaccharide material and calcium (Travis, 1951a). In the intermolt state the cells apparently store only polysaccharide (Fig. 4), whereas following molt they store calcium as small or large spheres and take on a "mulberry" appearance (Fig. 5). They may store fat as well. Although no material was fixed for the determination of the presence of lipid in the integumental tissues, similar cells in the connective tissue of the hepatopancreas contain much lipid. Cuénot (1893) described cells in the connective tissue of *Palinurus vulgaris* which stored large quantities of fat. His drawings indicate that these "fat cells" are similar in every respect to the reserve cells.

On first examination of the sub-epidermal tissues, it would appear that the reserve cells vary in number during various stages of the molting cycle, but this is not the case. When binding or storing reserves they are greatly increased in size, and their boundaries and nuclei are easily observed, especially with Helly's fixative and the PAS method. During periods when reserves are depleted, for example in late Stage C, many cells decrease in size and become clear and vesicular. Then cell boundaries are difficult to determine, but PAS and toluidine blue bring them out successfully.

In addition to the reserve cells (Type 1) there are: very small spindle cells with branching processes, *i.e.* Leydig cells, Type 2; rectangular cells (Fig. 6) which surround the walls of arteries, *i.e.*, Leydig cells, Type 3 (Kükenthal, 1926–1927); and amoebocytes in the blood spaces and channels of the connective tissue.

Tegumental glands were occasionally observed in the area from which these sections were cut. Collagen-like fibers are found throughout the connective tissue. At intervals within this tissue, the basal ends of some of the epidermal cells from each integument become long and attenuated and extend from one cuticle to the other (Fig. 7). The tonofibrillae (Fig. 7) which traverse the length of the epidermal cells are in close proximity to parallel collagen-like fibers which probably strengthen these supporting epithelial colonnades (Fig. 7) described by Vitzou (1882) in *Astacus fluviatilis* and *Homarus vulgaris*.

FIGURE 3. Photomicrograph of the integument of an intermolt animal (late State C). The four layers of the outer cuticle are represented by numbers. 1, epicuticle; 2, pigmented zone; 3, principal or calcified zone; 4, membranous layer. Note that outer integument is about 20 times thicker than inner cuticle. 90 ×.

FIGURE 4. Large, oval reserve cells (Leydig cells, Type 1) of the connective tissue with peripheral nuclei, one of which is indicated by arrow. Above arrow, collagen-like fibers found throughout the connective tissue. The small granules are glycogen (late Stage C). 450 ×.

FIGURE 5. The reserve cells take on an irregular "mulberry" appearance and their contents become divided up into small spheres as they store calcium following molt. 430 ×.

FIGURE 6. Large rectangular cells which surround the walls of arteries (Leydig cells, Type 3). The numeral (6) is within the lumen of an artery. 760 ×.

FIGURE 7. Colonnades of support, as indicated by arrow, are found at intervals throughout the connective tissue. 80 ×.

FIGURE 8. Tonofibrillae (T) which traverse the length of the epidermal cells. Arrow points to a pore canal, a protoplasmic extension of one of the epidermal cells. 1000 ×.

FIGURE 9. Photomicrograph showing very small amount of glycogen in the integumental tissues of an intermolt animal (late Stage C). Note small number of granules (a few are indicated at the tip of the arrow) localized between the reserve cells of the connective tissue. 450 ×.

FIGURE 10. Nuclei and cytoplasm of the integumental tissues are almost devoid of alkaline phosphatase during late Stage C. Nuclei indicated at tip of arrow show a positive reaction because of the presence of calcium. 80 ×.

b. *The Integument and its composition*

The outer integument, about 750 μ thick, consists of four distinct layers (Figs. 2, 3):

1. The most external layer, a thin epicuticle, is divided into two portions and is about 11 μ thick. It is composed of a tanned lipoprotein, and is impregnated with calcium salts.

2. A thicker layer, approximately 200 μ, is hardened by quinones as well as by calcium salts. This is called the pigmented layer of the outer endocuticle.

3. The third layer, about 460 μ in total thickness, is heavily calcified but non-pigmented and is called the principal layer or the calcified zone.

4. The fourth layer, approximately 90 μ in thickness, is non-calcified, non-pigmented, and is in direct contact with the underlying epidermis. This is called the membranous layer (Drach, 1939) or the non-calcified layer.

The thin inside cuticle next to the gill chamber is composed of two layers, a very thin epicuticle, and a uniformly staining endocuticle (Fig. 2).

Both the epicuticle and pigmented zone are formed before the molt. Drach (1939), therefore, prefers to call them pre-exuvial layers. He calls the principal layer and the membranous layer post-exuvial layers because of their formation after molt.

The basic components of the crustacean cuticle are, as in insects, chitin and protein, the properties of which may be altered by quinones, impregnation with lipids or calcium salts. Results obtained with toluidine blue would indicate that the principal and membranous layers of *Panulirus* contain a muco- or glycoprotein complex. These give gamma metachromasia (pink) while the pigmented layer shows beta metachromasia (violet). This difference is probably due to the fact that the properties of the protein and closely associated chitin units of the pigmented zone have been changed by quinones. Richards (1952) has similarly found that application of Schiff's reagent for histochemical demonstration of polysaccharides and glyco- or mucoproteins does not always give a positive reaction even though carbohydrate is present. He points out that the carbohydrate in the insect skeleton may be masked wherever sclerotization has occurred.

The horizontal laminations observed in all layers of the cuticle (Fig. 2) with the exception of the epicuticle, have been attributed by Drach (1939) to the rhythmical discharge of secretory material from the epidermal cells. Richards and Anderson (1942), however, suggest that these laminations may be the result of chemical changes in the constituents after secretion.

The vertical cross striae observed in sections of the cuticle are pore canals (Fig. 8), originally associated with protoplasmic extensions of the epidermis. The function of these is not entirely clear. Wigglesworth (1933, 1948) believes that they enable the epidermal cells to act at a distance upon the superficial layers of the cuticle and that hardening of the insect cuticle by quinones is effected through these canals. In *Panulirus argus,* hardening of the pre-exuvial layers by calcification would appear to be effected through these canals following molt (Travis, 1951a, 1951c).

c. *Localization of glycogen, phosphatase and calcium*

During late Stage C, there is little glycogen, phosphatase, and calcium evident in the integumental tissues. Glycogen when observed is sparsely localized between

the reserve cells or the connective tissues (Figs. 4, 9). Alkaline phosphatase appears to be completely lacking in the cytoplasm and nuclei of the integumental tissues. Some calcium, though sparse, is present in all nuclei (Fig. 10), occasionally present in distal ends of epidermal cells, and is infrequently bound in some polysaccharide complex in the few apparent reserve cells. All of the integument with the exception of the membranous layer is fully calcified.

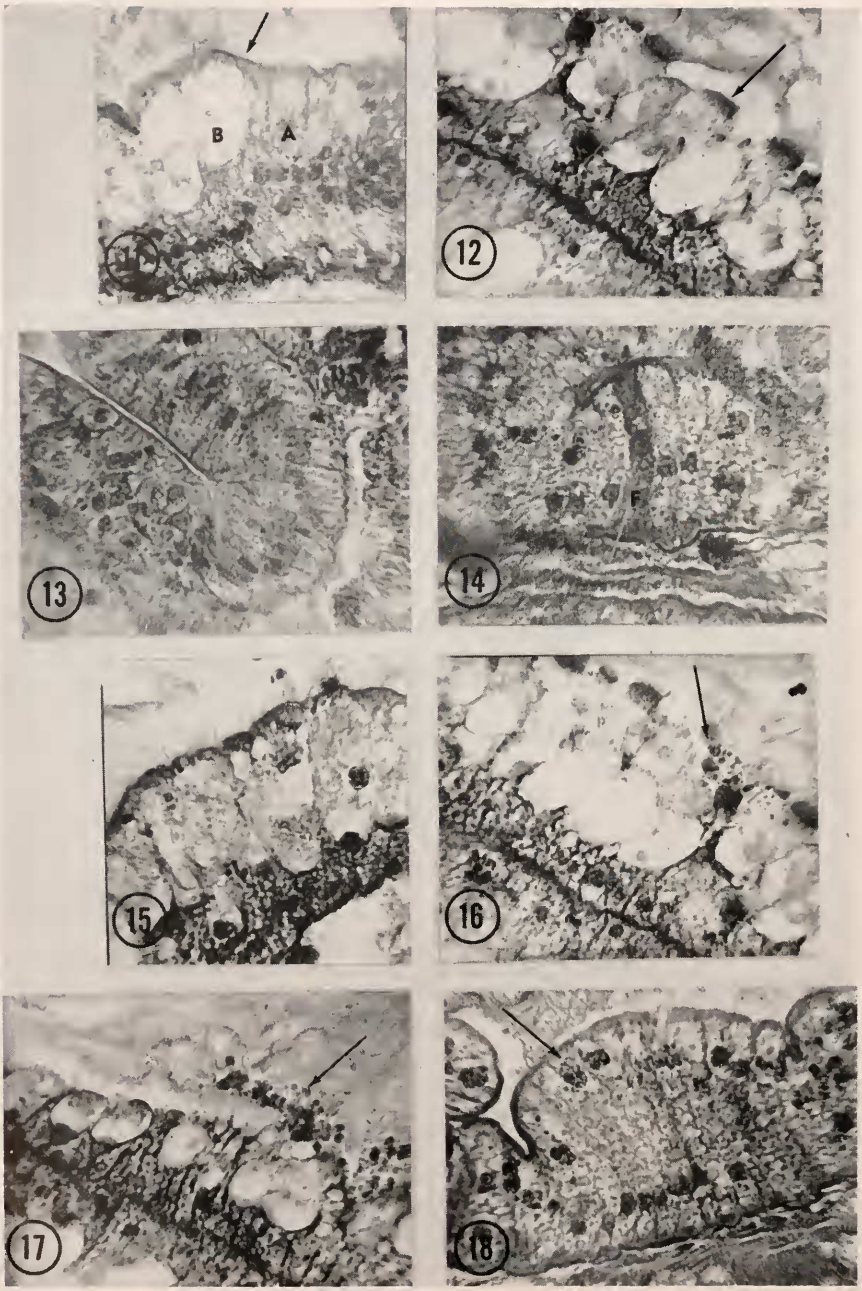
2. The hepatopancreas

a. *Tissues*

The hepatopancreas, a bilateral evagination of the midgut, functions in secreting digestive enzymes and absorbing and transforming food. It is also a major storage depot of organic and mineral reserves in Stage D and is, consequently, the major organ from which these reserves are mobilized when needed by other tissues. The gland itself is composed of innumerable tubules separated from each other by a loose connective tissue, which encloses large blood sinuses and branches of the hepatic arteries. There are collagen-like fibers intermingled between the large oval and vesicular reserve cells (as described in the discussion of the integumental tissues) which constitute the most numerous type of cells in the connective tissue. Amoebocytes are also present.

The tubular tissue proper is composed of columnar epithelium, delimited at its base by a basement membrane and characterized at its distal end (next to the lumen) by a striated border. The nuclei of these epithelial cells are basally located.

In an intermolt animal (late Stage C), a period during which active feeding occurs, the most conspicuous cells observed in the epithelial tissue of the hepatopancreas are large, mature, secretory cells (vesicular or B_2 cells of Hirsch and Jacobs, 1928, 1930). These are swollen, enclosing large vacuoles (Fig. 11), some of which contain stainable material. Their contents plus adjacent cytoplasm are discharged into the lumen, leaving only the basal region and nucleus of the cell intact (Figs. 12, 16, 17). Hirsch and Jacobs (1928, 1930) observed a complete breakdown of the secretory cells in *Astacus leptodactylus, i.e.,* holocrine secretion. Secretion in *Panulirus,* however, occurs in an apocrine rather than a holocrine fashion. The restitution of secretory cells in *Panulirus* is probably not nearly as complex as that observed in Crustacea where holocrine secretion takes place, and it is probable that these cells in *Panulirus* are reconstructed from the remaining basal end. Embryonic cells (E-cells, Fig. 13) predominate at the blind ends of the tubules. Hirsch and Jacobs (1928) pointed out in *Astacus leptodactylus* that these E-cells are followed proximally by B-cells and still more proximally by R-cells (Absorption cells). In *Panulirus* such a distinction is not so clear cut. Secretory cells and what would appear to be absorbing cells (Fig. 11) are frequently observed side by side. Fibrillar cells (Fig. 14) are not numerous, and these plus either absorbing or young secretory cells are scattered throughout the tubular tissue. Mature absorption cells (described in Kükenthal, 1926–1927) are tall columnar cells (Figs. 11, 14, 18, 19) with numerous small vacuoles and with either a basal or central nucleus, depending upon the stage of development. These would appear to be the same as the so-called B_1 cells of Hirsch and Jacobs. Whether the so-called absorption cells (R-cells) are really young secretory cells which have not developed large vacuoles or are special cells limited to absorption alone is not clearly evident.



FIGURES 11–18.

Mature B_2 or secretory cells as well as the tall columnar absorption-type cells contain both fat and glycogen. During Stage D, only the absorption cells contain large numbers of calcospherites. It is not impossible that in their life cycle the same cells perform both functions; absorption in their early stages and secretion in later stages. Certainly the epidermis of the integument performs both functions without the presence of any specialized types within it. In *Panulirus* the predominant hepatopancreatic epithelial cell types from specimens 1–5 days following molt are the long tall columnar cells without large vacuoles, the animal undergoing inanition during this period. Generally, feeding begins on the 6th or 7th day following molting. Because of the difficulty of distinguishing between absorption cells and young secretory cells (B_1-cells) described by Hirsch and Jacobs, and because of the possibility that these cells may perform absorption in their early stage of development and secretion in a later stage of development, the term secretory cell will be applied to those swollen cells containing very large vacuoles at their distal end and undergoing breakdown at the site of the large vacuole (Figs. 11, 12, 15, 16, 17). The name absorption cell will be applied to the tall cylindrical cells which do not contain large vacuoles at their distal end, but which may or may not have small vacuoles, and which may have either a central or basal nucleus (Figs. 11, 14, 18, 19). The presence of a central or basal nucleus may indicate a stage in the maturation of the cell. It will be observed that in Figures 11, 14 and 19 the nuclei of the absorption cells are centrally located, whereas those in Figure 18 are basally located. In the former cases, these cells may be younger than in the latter. In the latter case (Fig. 18), small vacuoles have developed around the calcospherites at the distal ends of the cells, and the nuclei have moved basally. These absorption cells may represent the oldest type before these same cells begin to perform secretion.

Figure 11. Portion of the tubular epithelium of the hepatopancreas showing (A) absorption type cells with central nuclei and (B) secretory cells containing large vacuoles. Nuclei in the latter are basally located. Note the striated borders, next to the lumen of the tubule, indicated by arrow. 430 ×.

Figure 12. Apocrine breakdown of secretory cells in the hepatopancreas showing ruptured vacuoles. Arrow points to the remnants of a striated border of a secretory cell. Note that the nuclei and basal ends of the secretory cells remain intact. 430 ×.

Figure 13. Section through blind end of a tubule of the hepatopancreas showing the numerous young embryonic cells (E-cells). 430 ×.

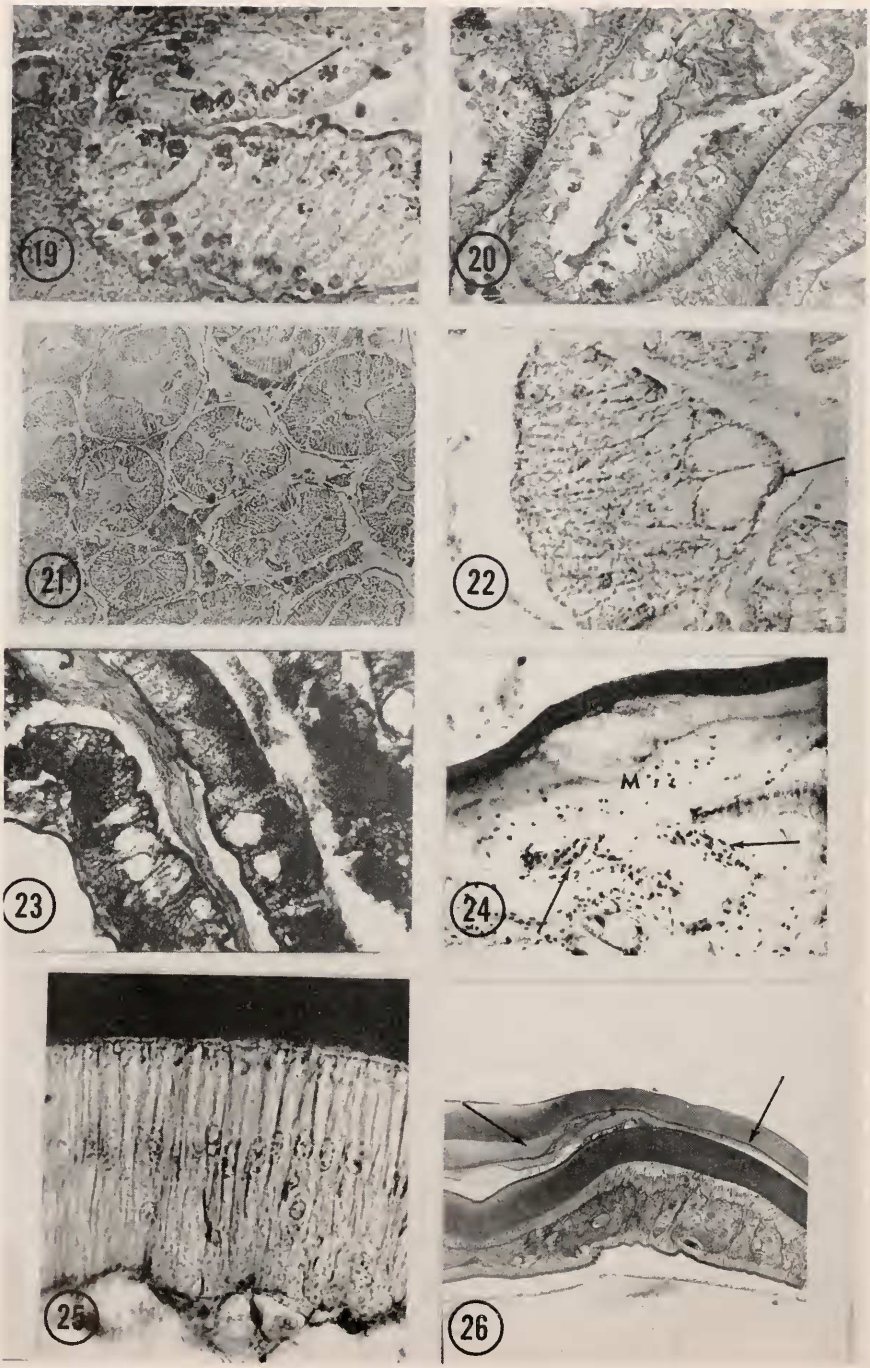
Figure 14. The fibrillar cells (F-cells) of Hirsch and Jacobs (1928, 1930). These are rare in the tubular tissue of the hepatopancreas of Panulirus. 430 ×.

Figure 15. Mature secretory cells of the hepatopancreas showing striated borders and basal nuclei. Note the secretory material within the vacuoles.

Figure 16. Secretory cells of the hepatopancreas which have undergone apocrine breakdown. Note that the basal ends of the cells containing the nuclei remain intact. The secretory granules (at end of arrow) and other contents of the vacuoles are discharged into the lumen of the tubule. Note remnants of striated borders of the secretory cells (to the left of the arrow). 430 ×.

Figure 17. Apocrine breakdown in secretory cells of the hepatopancreas. Note discrete secretory granules (at the end of the arrow) extruded into the lumen of the tubule. Basal ends of cells containing nuclei are preserved during secretory breakdown. 430 ×.

Figure 18. Old absorption cells of hepatopancreas with basal nuclei and numerous small vacuoles. The arrow points to calcospherites localized at the distal ends of these cells. Section from a premolt animal, fixed with Helly's and stained with Mallory's triple. 430 ×.



FIGURES 19–26.

b. *Localization of glycogen, phosphatase, calcium and lipid*

During late Stage C, the situation in the hepatopancreas is much the same as that observed in the integumental tissues. The few glycogen granules evident in secretory and absorbing cells (Fig. 20) are found at the bases of these cells and around the periphery of reserve cells in the connective tissue. Renaud (1949) demonstrated quantitatively in *Cancer pagurus* that little free glycogen could be detected in the hepatopancreas during most of Stage C because most appears to be utilized in the construction and growth of other tissues.

Alkaline phosphatase, present in small amounts, is localized at the striated borders of the epithelium and around the periphery of small and large secretory vacuoles (Figs. 21, 22). It would appear that the enzyme is strategically localized to participate in resorptive and secretory processes. Though the cell and nuclear membranes, and blood sinuses in the connective tissue give a positive reaction, control sections incubated without substrate indicate that most of this is due to the presence of calcium salts.

Calcium, though very sparse, is observed in all nuclei, cell membranes and in blood sinuses of the connective tissue. A very small amount is bound in complexes of the reserve cells.

Lipid is very abundant in the hepatopancreas during late Stage C. Large and small droplets are present throughout the epithelial tissues (both absorption and secretory cells, Fig. 23), and reserve cells; and they are frequently observed in the lumina of the tubules. Renaud (1949) has similarly observed numerous droplets of fat in the epithelial tissue of *Cancer pagurus* during this stage of the molting cycle.

FIGURE 19. Younger absorption cells, with basal nuclei, of a premolt hepatopancreas showing calcospherites at distal ends of cells. 430 ×.

FIGURE 20. Cross section of a tubule in the hepatopancreas of an intermolt animal (late Stage C) showing small amount of glycogen. Note that glycogen granules, although few in number, are distributed basally in both absorption and secretory cells (arrow). 100 ×.

FIGURE 21. Alkaline phosphatase distribution in the tubules of the hepatopancreas. 80 ×.

FIGURE 22. Portion of a single tubule of the hepatopancreas (late Stage C) at higher magnification to show that alkaline phosphatase is localized in the striated borders (at end of arrow) of the epithelium and around the periphery of small and large secretory vacuoles. 430 ×.

FIGURE 23. Section through hepatopancreas (late Stage C) to show the abundant distribution of lipid in both absorption and secretory cells of a single tubule. 80 ×.

FIGURE 24. A three-day premolt condition of the inner integument and epidermis (late Stage D). The epidermal cells have increased in number by undergoing extensive folding (not shown in this photograph). Some cells have disintegrated (arrows). Amoeboid cells appear in the molting fluid (M). 400 ×.

FIGURE 25. Outer integument and epidermis at three days preceding molt. Note that these outer epidermal cells have resumed an orderly alignment, have become much elongated and, in contrast to the situation observed in the inner integument and epidermis, have already secreted a portion of the new integument. 760 ×.

FIGURE 26. The condition of old and new integuments at about one or two days preceding molt. Note that the pre-exuvial layers of the new skeleton are almost fully formed, while resorption in the old skeleton is almost complete. Arrow to the left shows that about half of the old principal layer has been resorbed and arrow to the right indicates that all of the old skeleton, with the exception of the pigmented layer and epicuticle has been broken down by the molting fluid. These two latter layers are never attacked by the molting fluid. 90 ×.

B. THE PREMOLT ANIMAL (LATE STAGE D)

1. *External signs of the approaching molt*

The first distinct external evidence that *Panulirus* is approaching a molt is the appearance of a resorptive or ecdysial line (Travis, 1951a, 1954) along the branchiostegites. As Richards (1951) points out, along these lines sclerotization, indicated by lack of color, fails to occur. The ecdysial line in *Panulirus* appears as a result of marked resorption of organic and mineral constituents, thus leaving only a thin, translucent membrane. Histochemical tests have indicated that only the epicuticle and a thin mucilagenous layer remain, the latter being either the transformed membranous layer (Drach, 1939) or a transformed portion of the calcified zone. When resorption is complete in the endocuticle and when most of the calcium is resorbed from the epicuticle, some of these lines become weak and break, while others may act as hinges allowing flexibility and expansion of the soft body beneath the area in question (Herrick, 1895; Drach, 1939; Travis, 1951a, 1954).

In the entire area ventral to the resorptive or ecdysial line, less resorption occurs (20% resorption; Travis, 1951a) than in the line. The author calls this "the area of softening." In the summer months premolt animals can be detected as early as 5 days preceding ecdysis by feeling them along the area of softening. The resorptive line itself, however, is not clearly evident before 3–4 days preceding ecdysis.

2. *The integument and integumental tissues*

a. *Tissues and integument*

One of the most marked changes in the integumental tissues of a premolt animal is that observed in the epidermis. Approximately 10–14 days preceding molting in the summer months, detachment of the epidermis from the integument occurs, with a consequent development of a space between the two. This premolting stage was detected inadvertently on a number of occasions when secretory organs were removed from the eyestalks. Ten to fourteen days following epidermal retraction from the skeleton, the animals molted. Shortly following this retraction, growth of this tissue occurs. The mode by which growth is accomplished in the outer epidermis of the branchial region, whether by cell enlargement or by increase in cell number or both, has yet to be determined. The author has been unsuccessful so far in obtaining, for histological purposes, the precise stage at which the outer epidermis retracts and grows. This is difficult to obtain because by the time external signs of an approaching molt are obvious in the branchial region, the outside epidermis is past the retraction and growth stage and has begun to deposit the pre-exuvial layers. The epidermal cells of the inner cuticle increase in number causing a folding of the tissue. Some epidermal cells appear to disintegrate (Fig. 24). This is evident three days preceding molting. It would appear that processes leading to the growth of the epidermis, resorption from the old skeleton, and development of the new inside cuticle, all lag behind the growth of the epidermis, resorption from the old skeleton, and development of the new outside cuticle. This becomes evident from the following. At three days preceding ecdysis, nuclei, possibly of disintegrating cells of the inner epidermis and others of amoeboid cells, become trapped in what appears to be secreted molting fluid (Fig. 24). Yonge (1936) believes that these cells in *Homarus* attack and break down the chitin. At

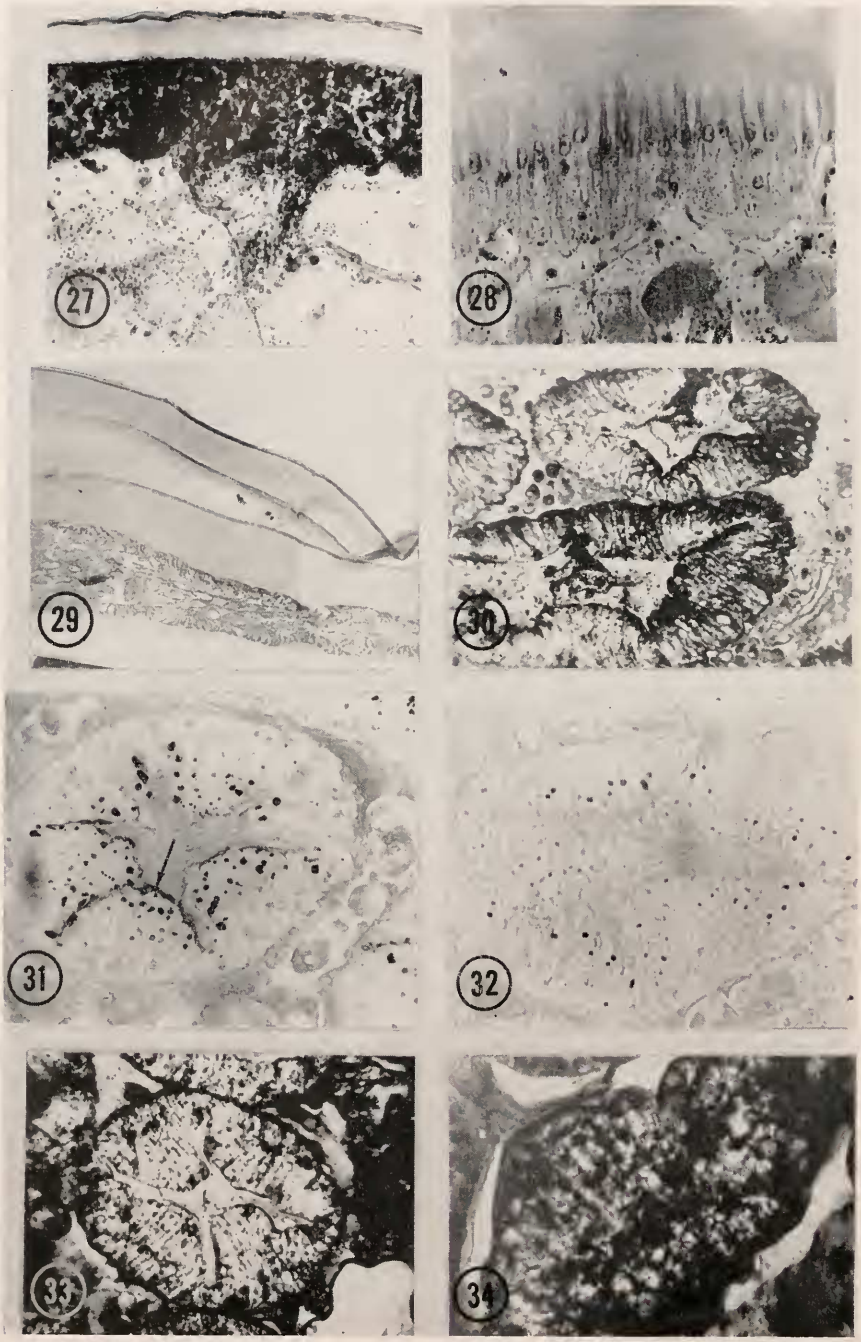
this same time, the epidermal cells of the outside cuticle have resumed an orderly alignment, have become very much elongated, fibrillar in nature, and have already secreted a portion of the new integument (Fig. 25). The new epicuticle of *Carcinus maenas,* shortly after its formation, is hardened by quinones (Krishnan, 1950, 1951). This also appears to be true of *Panulirus.* The thicker of the two pre-exuvial layers, however, becomes hardened by quinones following molt and is then called the pigmented layer. At one to two days preceding molt the old skeleton has thinned considerably (Fig. 26). It will be observed to the left of Figure 26 that almost half of the old principal layer has been resorbed, and to the right of this same figure all of the old skeleton, with the exception of the pigmented layer and epicuticle, has been broken down by the molting fluid. The molting fluid never attacks the latter layers. The immunity of the new epicuticle and pigmented layer to attack by the molting fluid is possibly due to the quinone tanning of the former. The quinone tanning of the epicuticle renders it highly stable and resistant to the enzymes of the molting fluid, but as Richards (1951) has pointed out, there is no adequate explanation of the failure of the newly forming procuticle to be digested by enzymes that must pass through it to reach the molting space.

The almost complete resorption, both mineral and organic, in some of the skeletal areas and partial resorption in others is absolutely necessary for complete separation of the new skeleton from the old and for allowing further thickening of the pre-exuvial layers before ecdysis. Due to resorption from the ecdysial line along the branchiostegites and resorption from an articulating condyle connecting the branchial chamber to the posterior edge of the branchiostegites, lateral expansion of the soft body beneath, and lifting of the old exuvia at molt is allowed (Travis, 1954). As resorption from the old skeleton and building of the new (pre-exuvial layers) progresses in the late premolt period, large numbers of reserve cells become apparent in the connective tissue. These are filled with polysaccharide complexes. This material probably represents not only breakdown products from the old skeleton but reserve substances for the new.

b. *Localization of glycogen, phosphatase and calcium*

During late Stage D, enormous amounts of glycogen are concentrated at the base and throughout the epidermal cells of the inner cuticle (Fig. 27), whereas very little is present in either the outer epidermis (Fig. 28) or the connective tissue. This condition persists until the second day following molt; glycogen then disappears from the inner epidermis for a seven-day period following molt. Little thickening occurs in the inside cuticle after one or two days postmolt, suggesting strongly that this cuticle is formed and completed early. The large amounts of glycogen observed in the inner epidermal cells during late Stage D and Stage A and its disappearance after this integument is completed would suggest that glycogen is a necessary precursor in chitin formation.

Alkaline phosphatase is observed in abundance at the distal ends of the epidermal cells which border on the cuticle (Fig. 29). Krugler and Birkner (1948) have similarly observed a heavy concentration at the distal ends of the epidermal cells of the integument of *Cambarus virilis.* The enzyme appears to be in both reserve cells and cells of Leydig (Type 3). Although these two latter sites are positive in the control sections, they are much lighter in appearance, indicating that the positive



FIGURES 27–34.

reaction of the reserve cells and cells of Leydig (Type 3) is probably due to the presence of calcium and not of the enzyme phosphatase. The enzyme doubtlessly participates in resorptive and secretory processes which are most pronounced at this time.

Although calcium is likewise localized at the distal ends of the outer epidermal cells and in blood sinuses beneath and between these cells, very little is bound in any of the reserve cells during Stage D. While calcium remains in portions of the old integument, *i.e.*, the epicuticle and pigmented layer, no calcification of the new skeleton, in the region of the branchiostegites, occurs until the second day following ecdysis.

3. *The hepatopancreas*

a. *Tissues*

During late Stage D, represented by animals approximately one to three days preceding molt, a marked difference is noted in the tubular epithelium of the hepatopancreas when compared with that of an animal in late Stage C. Likewise numerous reserve cells, filled with lipid and polysaccharide complexes, some of which contain bound calcium, are apparent in the connective tissue. The epithelium lining the tubules becomes high columnar and is composed predominantly of absorption-type cells. One striking difference between the epithelium of a premolt animal and that of an intermolt animal is the appearance of innumerable calcospherites in the distal ends (near the striated border) of the absorbing cells, while none is observed in the fibrillar, and very few are observed in the secretory cells. These calcospherites, spherules of calcium phosphate (previously observed in *Carcinus maenas* by Robertson, 1937), represent calcium salts withdrawn from the old skeleton, some of which are stored in the hepatopancreas during this period prior to molting. These stored calcium salts appear to be used in the hardening of the new skeleton, since calcification proceeds simultaneously with depletion of these stored reserves in the hepatopancreas (to be discussed in a subsequent paper). It is inter-

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FIGURE 27. One- to two-day premolt distribution of glycogen in the inner integument. Note the enormous amounts present in the epidermis while very little is evident in the connective tissue. 760 ×.

FIGURE 28. A one- to two-day premolt distribution of glycogen in the epidermis of the outer integument. Note that only very few granules are present. 450 ×.

FIGURE 29. A one- to two-day premolt distribution of alkaline phosphatase in the integumental tissues. The enzyme is heavily concentrated in the distal ends of the epidermal cells which border the integument. 90 ×.

FIGURE 30. The late Stage D distribution of glycogen in the tubular epithelium of the hepatopancreas. Note great concentrations of glycogen in the epithelial tissue and in the lumina of the tubules. 80 ×.

FIGURE 31. The late Stage D distribution of alkaline phosphatase in the hepatopancreas. A single tubule of this tissue is represented. Heavy localization, as indicated by arrow, in the striated borders of the absorbing cells and around the calcospherites. 100 ×.

FIGURE 32. A late Stage D control section of the hepatopancreas. Within a single tubule represented, only the calcium phosphate spherules remain visible following incubation without substrate. 100 ×.

FIGURE 33. The late Stage D distribution of lipid in the tubules of the hepatopancreas. 80 ×.

FIGURE 34. Portion of the epithelium of a single tubule of the hepatopancreas to show the heavy concentration of lipid droplets in the epithelium. 430 ×.

esting to point out that rather large amounts of calcium in the hepatopancreas have been detected by chemical analysis in *Carcinus maenas* by von Schönborn (1912) and Robertson (1937); in *Cancer pagurus* by Paul and Sharpe (1916); in *Maia squinado* by Drach (1939); and in *Hemigrapsus nudus* by Kincaid and Scheer (1952). All of these previous observations have been made on Brachyura. The observations on *Panulirus* indicate that calcospherites are also present in the hepatopancreas of Macrura.

b. *Localization of glycogen, phosphatase, calcium and lipid*

In late Stage D, glycogen, phosphatase, calcium, and lipid are markedly apparent in the hepatopancreas. Glycogen in abundance is observed in the epithelial tissue and even in the lumina of the tubules (Fig. 30). The connective tissue is, however, virtually devoid of it; glycogen is present in blood sinuses and around the periphery of reserve cells. It has previously been observed that during the premolt stage glycogen accumulates in the hepatopancreas of *Astacus fluviatilis* (Bernard, 1879; Vitzou, 1882; Kirch, 1886), *Homarus vulgaris* (Vitzou, 1882), and *Carcinus maenas* (von Schönborn, 1910). In addition to the increase of glycogen in the hepatopancreas before molt, it also appears in the integumental tissues of *Carcinus maenas, Cancer pagurus, Panulirus argus* and *Panulirus japonicus* (Verne, 1924, 1926; Renaud, 1949; Travis, 1951a; and Schwabe *et al.*, 1952). It will be recalled that the integumental tissues of *Panulirus argus* during this same period begin to accumulate large amounts. These stores, initially accumulated by the hepatopancreas, are mobilized and transported to the integumental tissues for the synthesis of the skeleton.

Alkaline phosphatase is heavily concentrated in the striated borders of absorbing cells (Fig. 31). The most striking localization is that observed around innumerable calcospherites, which are sites of calcium phosphate deposition in the distal ends of the absorbing cells (Fig. 31). This very strongly suggests that alkaline phosphatase is directly participating in the deposition of calcium phosphate calcospherites. The secretory cells have few calcospherites and show very little evidence of phosphatase in their striated borders. Thus the enzyme and calcospherites appear to be predominantly localized in absorption-type cells. Though the reserve cells show darkening, control sections indicate that this darkening is due primarily to calcium which is bound in lipid and polysaccharide complexes and not to the enzyme phosphatase. Calcium is also present in blood sinuses within the connective tissue and is localized in cell and nuclear membranes of all cells.

Droplets of fat are found throughout the epithelial tissue (Figs. 33, 34). There is little change over that observed during late Stage C. Where secretory cells are observed, droplets of fat are found throughout these cells, and much of the material in the large vacuoles is lipid. Fat is also abundant in the reserve cells of the connective tissue. Renaud (1949) noted that lipids of the hepatopancreas in *Cancer pagurus* disappear little by little during the fasting period but are still abundant in late Stage D through Stage B, then undergo a marked decrease. The hepatopancreas serves, among other functions, as an important storage depot of fat. Cuénot (1893) was one of the first to indicate this in *Astacus fluviatilis*. In *Cancer pagurus, Lithodes*, and *Homarus* (Paul and Sharpe, 1916) and in *Cancer pagurus* (Renaud, 1949) there is an accumulation of lipids in the hepatopancreas

preceding molt and a gradual disappearance following molt. Likewise Damboviceanu (1932) showed that blood fatty acids increase before molt in *Astacus fluviatilis* and slowly fall following molt and reach normal levels when the skeleton fully hardens.

Thus during Stage D the hepatopancreas becomes an important storage organ for fat, glycogen and calcium reserves. These, as will be pointed out in a subsequent paper, disappear progressively as growth of the integument and other tissues occurs.

Discussion

External signs of an approaching molt in *Panulirus* reflect profound physiological transformations which occur in the tissues of the organism. These changes manifest themselves in the epidermis and sub-epidermal tissues of the integument as well as the epithelial and connective tissues of the hepatopancreas. All of these changes during the pre-ecdysial period are associated with the growth of the organism. The structure restricting the growth in size to certain periods throughout the year is the rigid exoskeleton. A study, therefore, of changes which occur in the integumental tissues, and changes which effect breakdown of the old skeleton and growth of the new, cannot be considered apart from the more general aspects of growth and molting in the arthropods.

During the intermolt period (late Stage C) the skeleton of the branchial region in *Panulirus* is fully hardened and consists of four distinct layers, the epicuticle, the pigmented layer, the principal layer, and the membranous layer. The first three layers are calcified, whereas the fourth is non-calcified. In the Crustacea, the epicuticle and pigmented layer, in addition to being calcified, are hardened by quinones (Dennell, 1947; Kirshnan, 1950, 1951, 1954). In crustaceans as in insects the basic components of the skeleton are chitin and protein which are firmly associated with one another. Trim (1941), Stacy (1943) and Haworth (1946) regard the arthropod cuticle as a mucopolysaccharide because of the firm combination of the carbohydrate containing amino sugars (chitin) with the proteins. Glycogen, phosphatase and calcium are three necessary constituents, therefore, to consider during the breakdown of the old and the growth of the new skeleton.

During late Stage C, no marked changes occur in the hepatopancreas or in the integumental tissues. Few reserve cells are apparent either in the connective tissue of the liver or in subepidermal tissues of the integument. In both areas, such cells contain little carbohydrate, lipid, and calcium. Even though the animals actively feed throughout this period, glycogen is low in both the hepatopancreas and integumental tissues. This is probably the result of its utilization in the growth of other tissue. Renaud (1949), and Schwabe *et al.* (1952), noted similar situations in *Cancer pagurus* and *Panulirus japonicus,* respectively. There is also little evidence of the enzyme phosphatase in the integumental tissues. It is present, however, where active absorption and secretion occur in the hepatopancreas. Calcium is found in the usual locations, *i.e.,* in all nuclei and cell membranes of the integumental and hepatopancreatic tissues and in blood sinuses in the connective tissues. At this period large amounts of fat are stored in the hepatopancreas.

As the pre-ecdysial period (Stage D) is approached, marked changes are observed in the integumental tissues and the skeleton. Most profound of the internal morphological changes are those in the epidermis. In the summer months it is

caused to retract from the old skeleton 10–14 days preceding the molt, so that a molting space develops between the skeleton and retracted tissue. Following this retraction, growth of the epidermis occurs. The mode by which growth occurs has not been observed in the outer epidermis of the branchial region; however, the epidermal cells of the inner integument appear to increase in number causing a folding of the tissue. Such a condition appears three days before molt, indicating that processes which lead to growth of the epidermis, breakdown of the old skeleton, and growth of the new skeleton bordering the gill chamber, lag behind those processes which occur in the outer integument. While growth of the epidermis and breakdown of the old skeleton of the inside integument occur, the epidermal cells of the outer integument have already resumed an orderly alignment, have become very much elongated, and have secreted a portion of the new skeleton (epicuticle and pigmented layer). At one to two days preceding molt, almost all resorption in the old outside skeleton of the branchial region is complete. Mineral and organic resorption from the skeleton—complete in some areas, partial in others—is necessary to free the new skeleton from the old and to allow expansion of the soft body and further thickening of the pre-exuvial layers before ecdysis (Travis, 1954). During this premolt period, when resorption and growth of the new skeleton are occurring simultaneously, large numbers of reserve cells stand out because they are filled with a polysaccharide complex, which may represent either breakdown products from the old skeleton or reserve materials for the new. At this stage, very little calcium is bound to the polysaccharide complex. During the period, large amounts of glycogen are observed in the hepatopancreas. These glycogen granules are localized at the basal and distal ends of the absorbing cells and are observed frequently within the lumina of the tubules. Integumental glycogen comes largely from the hepatopancreas. Since the animals do not feed during this period, much of the glycogen may come from the conversion of fats stored in the hepatopancreas. Large amounts of glycogen are also concentrated throughout the epidermal cells of the inner integument, while very little is evident in either the connective tissue or the outer epidermis. This condition persists until the second day following molt, at which time the glycogen disappears from the epidermis of the inner integument. As the principal layer of the outer integument is deposited following molt, glycogen is observed to accumulate in the outer epidermis. There is a periodic shift of glycogen from the connective tissue to the outer epidermis (Travis, 1951a, 1951c) which probably indicates that this tissue goes through rhythmical periods of accumulation and utilization as the post-exuvial layers are deposited. This evidence suggests strongly that glycogen is a necessary precursor for chitin formation and is needed not only for this synthesis but for growth of other tissues as well. The fact that glycogen disappears from the inner epidermis on the second day following molt and that little thickening of the inner integument occurs after this period, suggests that the inner integument is completed during a period of three days preceding molt and two days following molt. Large amounts of glycogen, therefore, appear to be utilized at this time by the inner epidermal cells for the synthesis of chitin. That glycogen is a necessary precursor in the synthesis of chitin has also been suggested by Verne (1924, 1926), Renaud (1949), Travis (1951a), Schwabe *et al.* (1952). Renaud (1949) has further proposed a general mechanism by which glycogen may be utilized as a precursor in chitin formation. She suggests that glycogen is first hydrolyzed to glucose, and that this is aminated,

yielding glucosamine which, after acetylation to N-acetyl-glucosamine, undergoes polymerization to yield chitin. During the pre-ecdysial period, hydrolysis of glycogen would contribute greatly to the high blood glucose noted in *Callinectes sapidus* by Baumberger and Dill (1928), in *Astacus fluviatilis* by Damboviceanu (1932), and in *Maia squinado* by Drilhon (1933, 1935). Scheer and Scheer (1951) found that glucose apparently does not serve as a substrate for oxidative metabolism in *Panulirus japonicus* and that it does not stimulate the consumption of O_2 in isolated tissues, since little labeled C^{14} (in injected glucose) was recovered in respiratory CO_2. They further support the idea that the glycogen, present in the hepatopancreas and other tissues, is converted to glucose and that its principal role in the Crustacea is in the formation of chitin. It is interesting that high blood glucose may also be accompanied by increases in organic acids and other intermediates of glycolytic metabolism, thus contributing to the increase in osmotic pressure and facilitating water intake during and following molt. A further, possibly important, function of glycogen during the pre-ecdysial period is an intimate involvement in the deposition of calcospherites in the hepatopancreas. Moog (1946) has suggested the importance of glycogen in calcification of bone. With the abundance of glycogen in the hepatopancreas during Stage D and its possible hydrolysis and subsequent phosphorylation, glycogen could serve indirectly as added substrate (phosphoric esters) for phosphatase action, which, as will be pointed out subsequently, appears to be intimately concerned with the deposition of calcium phosphate calcospherites at the periphery of absorbing cells in the hepatopancreas.

During late Stage D, phosphatase appears in the epidermis and connective tissue. It is localized at the distal ends of the epidermal cells bordering the integument, is present in the reserve cells, and in blood sinuses of the connective tissue. Phosphatase present in these strategic sites of active transfer of materials throughout the integumental tissues, probably participates in those reactions involving hydrolysis and dephosphorylation of glucose phosphate to glucose, which Renaud (1949) suggested to be a probable starting point for chitin formation. Hydrolysis of phosphoric esters would produce molecules which would be able to enter or leave these tissues more readily. The enzyme does not participate in calcification of the branchial integument during the premolt period, since no calcification occurs at this time, although calcium is localized at the distal ends of the epidermal cells of the outer integument and in blood sinuses beneath and among the epidermal cells. In the hepatopancreas alkaline phosphatase is heavily concentrated at the striated borders of absorbing cells. The enzyme is most concentrated around calcium phosphate deposition sites (calcospherites) which are predominantly localized in the distal ends of the absorbing cells. Phosphatase in the hepatopancreas appears not only to be involved in important transfer and dephosphorylation reactions which occur at the surface of the absorbing cells but also to be intimately concerned with the deposition of calcium phosphate calcospherites at the periphery of these same cells. Here the enzyme possibly acts by catalyzing local liberation of phosphate ions from the organic phosphates present in the blood or from those transferred across the cell membranes. Calcium ions which are provided by the blood and which are transferred across the cell membranes then unite with these freed phosphate ions to form deposited calcospherites. It may be pointed out that during this period blood calcium rises from an average intermolt value of 22 mEq/L to a peak premolting average of 41 mEq/L and that total blood phosphorus rises from

3.6 to 5.3 mEq/L. However, little change is observed in blood inorganic phosphate, which remains within the normal intermolt range of 0.28 mEq/L (Travis, 1951a, 1951b). Glycogen, it will be recalled, is abundant in these same tissues and provides a source of phosphoric esters which act as substrate for phosphatase activity. Since the animals do not feed for two weeks preceding and one week following molt, and since the primary source of phosphate is from food (Travis, 1951a, 1951b), all available phosphate must be conserved. The calcospherites represent a means of storing or conserving resorbed phosphate from the old skeleton. Although this will be discussed in a subsequent paper, it may be said that following ecdysis the calcospherites disappear from the hepatopancreas as growth and mineral deposition occurs in the new skeleton.

In addition to the great quantities of glycogen, calcospherites, and heavy localization of phosphatase, the hepatopancreas stores large amounts of lipid during Stages D, A, and B. Other lipid deposits occur around the supra- and sub-esophageal ganglia. It will be recalled that during Stages D, A, and B of the molting cycle, the animals do not feed. It is possible that during such periods of inanition the animals utilize their fat reserves as a major source of energy. This is evident from the work of Renaud (1949) who showed that, although a fraction of protein nitrogen of the hepatopancreas is utilized as an energy reserve during the period of starvation, the reserves that permit *Cancer pagurus* to subsist during this period are constituted mostly from fatty acids and glycerides. Bliss (1952, 1953) has shown that fat and protein appear to be the principal foods oxidized even in normal intermolt *Gecarcinus,* as indicated by the mean respiratory quotient values of 0.77. It would seem, therefore, that during periods of fasting the stores of fat in the hepatopancreas serve as a major source of energy and that they play a principal role in oxidative metabolism of the normal intermolt animal.

The large amounts of fat stored in the hepatopancreas during the pre-ecdysial period may contribute indirectly, through their metabolism, to the rise in blood osmotic pressure preceding molt, which facilitates water intake during and immediately following molt. That fats (fatty acids and glycerols) are present in large quantities within the hepatopancreas and blood preceding molt and that they slowly decline until feeding begins following molt (Stage C) is shown by a number of investigations. Paul and Sharpe (1916) found that prior to molt in *Cancer pagurus, Lithodes,* and *Homarus* there was increased fat storage (fatty acids and glycerides) in the hepatopancreas. Following molt, fatty acids and glycerides decreased in the hepatopancreas and increased in the blood. Damboviceanu (1932) demonstrated a rise in blood fatty acids of *Astacus* before molt, followed by a slow decline during the postmolt period. Drilhon (1935) showed an augmentation before molt of total lipids in the blood, hepatopancreas, and in genital organs of *Maia squinado.* This rise was followed by a fall during and following molt. Renaud (1949) found that total lipids, fatty acids, phosphatides, cholesterol and total unsaponifiable fat increase to a peak in the hepatopancreas preceding molt, slowly fall following molt, and reach their lowest point as the skeleton is hardened (Stage C). Bliss (1952, 1953) reported that eyestalkless *Gecarcinus* approaching molt have an R.Q. greater than one, which to her suggested metabolism of organic acids. It is highly likely that such a situation may occur in normal animals approaching molt. This is very interesting because it would seem from the evidence available that the increase in blood fatty acids, phosphatides, cholesterol and glycerides, as well as organic acids which ac-

cumulate during glycolysis, might very well contribute, as Baumberger and Olmsted (1928) pointed out, to the rise in blood osmotic pressure preceding molt which would facilitate water absorption shortly preceding and following molt.

The fatty acids and cholesterol conveyed to the integumental tissues preceding molt are without doubt used in the formation of the epicuticle. Renaud (1949) showed that in *Cancer pagurus* immediately before formation of the epicuticle, the amount of lipids (fatty acids, cholesterol and unsaponifiable fatty acids) in the epidermis rises from 19% in late Stage C to 33% in Stage D when the epicuticle is being formed. After the epicuticle is formed, the hypodermis is emptied of 1/3 of its lipids.

It is pertinent to suggest the importance of many of these organic acids, products of oxidative and glycolytic metabolism, as carriers of calcium. Such carriers play a part in conveying calcium to the hepatopancreas for storage preceding molt and likewise in conveying it to the integumental tissues as the skeleton progressively calcifies following molt.

It is evident from the foregoing discussion that the marked histological and histochemical changes which occur in the hepatopancreas and integumental tissues during the pre-ecdysial period are associated with the growth of the organism. The hepatopancreas participates intimately in the important processes related to growth of the epidermis. Because it is a major storage depot for glycogen, fat and mineral reserves (calcium phosphate calcospherites) during the pre-ecdysial period, the hepatopancreas is the major organ from which these reserves are mobilized when needed in growth of the integument and other tissues. The possible functions of these reserves in such processes have been suggested.

Summary

1. The integumental tissues from the branchial region of an intermolt spiny lobster (late Stage C) are bordered by an outer and an inner integument. The outer integument consists of four layers: a thin epicuticle, the pigmented layer, the principal layer, and the membranous layer. Both the epicuticle and pigmented layer are formed before molt. The inner integument, about 1/20 the thickness of the outer integument, is composed of two layers, a very thin epicuticle and a uniformly staining endocuticle.

2. The integumental tissues are composed of columnar epidermal cells and a sub-epidermal connective tissue of a loose spongy type. Large oval reserve cells constitute by far the most predominant type of cells in this tissue. These cells store abundant amounts of carbohydrate, lipid and calcium at various stages throughout the molting cycle; they are also found in the hepatopancreas.

3. Within the tubular epithelium of the hepatopancreas are two major types of cells, secretory and absorptive. The former are swollen cells containing very large vacuoles at their distal ends. Secretion is of the apocrine type, leaving only the basal region and nucleus of the cell intact. Reconstitution of secretory cells probably occurs by regeneration from the remaining basal ends. The latter type of cells (absorptive) are tall and cylindrical with a basal or central nucleus.

4. During the intermolt period (late Stage C), a period of active feeding, there is little glycogen, phosphatase, and calcium evident in either the hepatopancreas or the integumental tissues. Lipids, however, are abundant in the epithelial tissues of the hepatopancreas.

5. External signs of an approaching molt (Stage D) become evident by the appearance of a resorptive or ecdysial line along the branchiostegites 3–4 days preceding molting in the summer.

6. The external signs of an approaching molt in *Panulirus* reflect the more basic internal changes which occur in the integumental tissues and hepatopancreas. Growth of the outer epidermis occurs shortly after its retraction from the outer integument, 10–14 days preceding molt. Growth of the epidermis of the inside integument is accomplished through an increase in cell number at 3 days preceding molt. Resorption from the old and completion of the new inner integument occurs in a period of five days, three days preceding and two days following molt. At three days preceding molt the epidermal cells of the outer integument have already resumed an orderly alignment, are greatly elongated, and have secreted the epicuticle and pigmented layer of the new skeleton. By this time most of the principal layer of the old outer integument has been broken down by the molting fluid. The almost complete mineral and organic resorption in some areas, partial in others, allows for complete freeing of the new skeleton from the old and for further thickening of the pre-exuvial layers before ecdysis.

7. During the pre-ecdysial period (late Stage D) large numbers of reserve cells of the integumental tissues become greatly swollen, and bind or store large amounts of polysaccharide material. Glycogen is abundant in the epithelial tissues of the hepatopancreas and simultaneously accumulates in the epidermal cells of the inner integument as the latter is being formed. Available evidence (see discussion) would suggest strongly that glycogen is a necessary precursor for chitin formation. Furthermore, glycogen is perhaps intimately involved in the deposition of calcospherites in the hepatopancreas.

8. During late Stage D, phosphatase appears in the distal ends of the epidermal cells bordering the integument and in reserve cells of the connective tissue. It is suggested that phosphatase in these strategic sites of active transfer probably participates in producing molecules which are able to enter or leave the cells more readily, and in those reactions involving hydrolysis and dephosphorylation of glucose phosphate to glucose, a possible starting point for chitin formation. It is not participating in calcification of the branchial integument at this time, because calcification does not begin until the second day following molt. In the hepatopancreas, on the other hand, alkaline phosphatase is heavily concentrated at the striated border of the absorbing cells and around calcium deposition sites (calcospherites). Here the enzyme appears to be involved in important transfer and dephosphorylation reactions which occur at the surface of the absorbing cells and also to be intimately concerned with the deposition of calcium phosphate at the periphery of these same cells (see discussion).

9. Evidence available suggests that lipids, abundant in the hepatopancreas at this time, function as a major source of energy. Furthermore, the accumulation of fatty acids, glycerides, and other intermediates of oxidative as well as glycolytic metabolism during late Stage D and Stage A may contribute to the rise in osmotic pressure preceding molt, facilitating water intake. Further evidence suggests that some of the fatty acids, cholesterol and unsaponifiable fatty acids conveyed to the integumental tissues preceding molt are used in the formation of the epicuticle.

10. It is suggested that many of the organic acids, products of oxidative and glycolytic metabolism, are perhaps important carriers of calcium. Such carriers

participate in conveying calcium to the hepatopancreas for storage preceding molt, and in conveying it to the integumental tissues as the skeleton progressively calcifies following molt.

LITERATURE CITED

Baumberger, J. P., and D. B. Dill, 1928. Study of glycogen and sugar content and the osmotic pressure of crabs during the molt cycle. *Physiol. Zool.*, **1**: 545–549.

Baumberger, J. P., and J. M. D. Olmsted, 1928. Changes in osmotic pressure and water content of crabs during the molt cycle. *Physiol. Zool.*, **1**: 531–543.

Bensley, R. R., and S. H. Bensley, 1938. Handbook of histological and cytochemical technique. Univ. of Chicago Press, Chicago.

Bernard, C., 1879. Leçons sur les phénomènes de la vie communs aux animaux et aux végétaux., II: 1–564. Baillière, Paris.

Blank, H., and P. L. McCarthy, 1950. A general method for preparing histologic sections with a water soluble wax. *J. Lab. Clin. Med.*, **36**: 776–781.

Bliss, D. E., 1952. Endocrine control of metabolism in the decapod crustacean, *Gecarcinus lateralis*. Thesis for Ph.D. degree, Radcliffe College.

Bliss, D. E., 1953. Endocrine control of metabolism in the land crab, *Gecarcinus lateralis* (Fréminville). I. Differences in the respiratory metabolism of sinusglandless and eyestalkless crabs. *Biol. Bull.*, **104**: 275–296.

Bronn, H. G., 1896–1907. Klassen und Ordnungen des Tierreichs, **3**: 292–294, 805–806.

Cuénot, L., 1893. Études physiologiques sur les crustacés décapods. *Arch. de Biol.*, **13**: 245–303.

Damboviceanu, A., 1932. Composition chimique et physico-chimique du liquide cavitaire chez les crustacés decapods. (Physiologie de la calcification.) *Arch. Roum. Path. Exp. et Microbiol.*, **5**: 239–309.

Dennell, R., 1947. The occurrence and significance of phenolic hardening in the newly formed cuticle of Crustacea decapods. *Proc. Roy. Soc. London, Ser. B*, **134**: 485–503.

Drach, P., 1939. Mue et cycle d'intermue chez les crustacés décapods. *Ann. Inst. Oceanogr.*, **19**: 103–391.

Drilhon, A., 1933. La glucose et la mue des crustacés. *C. R. Acad. Sci.*, **196**: 506–508.

Drilhon, A., 1935. Étude biochimique de la mue chez les crustacés. *Ann. Physiol. et Physicochem. Biol.*, **11**: 301–326.

Gomori, G., 1941. The distribution of phosphatase in normal organs and tissues. *J. Cell. Comp. Physiol.*, **17**: 71–83.

Haworth, W. N., 1946. The structure, function and synthesis of polysaccharides. *Proc. Roy. Soc. London, Ser. A*, **186**: 1–19.

Herrick, F. H., 1895. The American lobster. *Bull. U. S. Fish Comm.*, **15**: 1–252.

Hirsch, G. C., and W. Jacobs, 1928. Der Arbeitsrhythmus der Mitteldarmdrüse von *Astacus leptodactylus*. Part I. Der Bewis der Periodizitat. *Zeitschr. vergl. Physiol.*, **8**: 102–144.

Hirsch, G. C., and W. Jacobs, 1930. Part II. Wachstum als primarer Faktor des Rhythmus eines polyphasischen organigen Sekretions-system. *Zeitschr. vergl. Physiol.*, **12**: 524–558.

Kincaid, F. D., and B. T. Scheer, 1952. Hormonal control of metabolism in crustaceans. IV. Relation of tissue composition of *Hemigrapsis nudus* to intermolt cycle and sinus gland. *Physiol. Zool.*, **25**: 372–386.

Kirch, J. B., 1886. Das Glycogen in den Geweben des Flusskrebses. Inaug. Diss Bonn. As quoted from Cuénot, 1893.

Krishnan, G., 1950. Sinus gland and tyrosinase activity in *Carcinus maenas*. *Nature*, **165**: 364–365.

Krishnan, G., 1951. Phenolic tanning and pigmentation of the cuticle in *Carcinus maenas*. *Quart. J. Micr. Sci.*, **92**: 333–342.

Krishnan, G., 1954. Tyrosinase activity in relation to phenolic tanning of the cuticle in *Carcinus maenas*. *Proc. Nat. Inst. Sci. India*, **20**: 157–169.

Krugler, O. E., and M. L. Burkner, 1948. Histochemical observations of alkaline phosphatase in the integument, gastrolith sac, digestive gland and nephridium of the crayfish. *Physiol. Zool.*, **21**: 105–110.

Kükenthal, W., 1926–1927. *Handbuch der Zoologie,* **3**: 848–849, 888–889.
Lillie, R. D., 1948. Histopathologic technic. The Blakiston Co., Philadelphia.
Manigault, P., 1939. Recherches sur le calcaire chez les mollusques. Phosphatase et précipitation calcique. Histochimie du calcium. *Ann. de Inst. Oceanogr.,* **18**: 331–425.
Meyer, K., 1938. The chemistry and biology of mucopolysaccharides and glycoproteins. *Cold Spring Harbor Symp.,* **6**: 91–101.
Moog, F., 1946. The physiological significance of the phosphomonesterases. *Biol. Rev.,* **21**: 41–59.
Paul, J. H., and J. S. Sharpe, 1916. Studies on Ca metabolism. I. The deposition of lime salts in the integument of decapod Crustacea. *J. Physiol.,* **50**: 183–192.
Pearse, A. G. E., 1953. Histochemistry theoretical and applied. Little Brown and Company, Boston.
Renaud, L., 1949. Le cycle des réserves organiques chez les crustacés Décapodes. *Ann. Inst. Oceanogr.,* **24**: 260–357.
Richards, A. G., 1951. The integument of arthropods. Univ. of Minnesota Press, Minneapolis.
Richards, A. G., 1952. Studies on arthropod cuticle. 7. Patent and masked carbohydrate in the epicuticle of insects. *Science,* **115**: 206–208.
Richards, A. G., and T. F. Anderson, 1942. Electron microscope studies of insect cuticle. *J. Morph.,* **71**: 135–183.
Robertson, J. D., 1937. Some features of Ca metabolism of the shore crab (*Carcinus maenas* Pennant). *Proc. Roy. Soc. London, Ser. B,* **124**: 162–182.
Scheer, B. T., and M. A. R. Scheer, 1951. Blood sugar in spiny lobsters. Part I of the hormonal regulation of metabolism in crustaceans. *Physiol. Comp. et Oceol.,* **2**: 198–209.
Schönborn, E. G. von, 1910. Beitrage zur Kenntnis Kohlenhydratstoffwechsels bei *Carcinus maenas. Zeitschr. f. Biol.,* **55**: 70–82.
Schönborn, E. G. von, 1912. Weitere Untersuchungen über den Stoffwechsel der Krustazeen. *Zeitschr. f. Biol.,* **57**: 534–544.
Schwabe, C. W., B. T. Scheer and M. A. R. Scheer, 1952. The molt cycle in *Panulirus japonicus.* Part II of the hormonal regulation of metabolism in crustaceans. *Physiol. Comp. et Oecol.,* **2**: 310–320.
Scott, G. H., 1933. The localization of mineral salts in cells of some mammalian tissues by microincineration. *Amer. J. Anat.,* **53**: 243–287.
Stacy, D. M., 1943. Mucopolysaccharides and related substances. *Chem. Ind.,* **62**: 110–112.
Travis, Dorothy F., 1951a. Calcium metabolism in the decapod Crustacea. Thesis for Ph.D. degree, Radcliffe College.
Travis, Dorothy F., 1951b. Physiological changes which occur in the blood and urine of *Panulirus argus* Latreille during the molting cycle. *Anat. Rec.,* **111**: 157.
Travis, Dorothy F., 1951c. Early stages in calcification of the skeleton of *Panulirus argus* Latreille. *Anat. Rec.,* **111**: 124.
Travis, Dorothy F., 1954. The molting cycle of the spiny lobster, *Panulirus argus* Latreille. I. Molting and growth in laboratory-maintained individuals. *Biol Bull.,* **107**: 433–450.
Trim, A. R., 1941. Studies in chemistry of the insect cuticle. I. *Biochem. J.,* **35**: 1088–1098.
Verne, J., 1924. Note histochimique sur le métabolisme du glycogène pendant la mue chez les Crustacés. *C. R. Soc. Biol.,* **90**: 186–188.
Verne, J., 1926. L'édification de la carapace chitineuse chez les crustacés Décapodes. *C. R. Assoc. Anat.,* **21**: 551–556.
Vitzou, A. N., 1882. Recherches sur la structure et la formation des téguments chez les Crustacés décapodes. *Arch. Zool. Exp. et Gen., Series I,* **10**: 451–576.
Wigglesworth, V. B., 1933. The physiology of the cuticle and of ecdysis in *Rhodnius prolixus* with special reference to the function of the oenocytes and the diurnal glands. *Quart. J. Micr. Sci.,* **76**: 269–318.
Wigglesworth, V. B., 1948. The insect cuticle. *Biol. Rev.,* **23**: 408–451.
Yonge, C. M., 1936. On the nature and permeability of chitin. II. The permeability of uncalcified chitin lining the foregut of *Homarus. Proc. Roy. Soc. London, Ser. B,* **120**: 15–41.